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Influence of IV domain apical loop GNRA-alterations on IRES-driven translation in circular RNA-vectors

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In recent years vectors based on circular RNA has become a powerful instrument for vaccines production. Comparing to linear vectors, circular RNA (circ-RNA) offers long-lasting expression.

Internal ribosome entering site (IRES) plays a main role in translation of circ-RNA and only type I IRESes suit for it. This group contains Poliovirus IRESes including Coxsackie virus B3 and some Rhinoviruses[1]. The understanding of molecular mechanisms laying in the basement of IRES-driven translation is important for further development circular RNA-vectors.

IRES structure is highly conservative: numerous works claims, that deletions of domains or hidden motives, such as GNRA, different insertions causing disruption of secondary structure leads to total loss of IRES activity[1]. That is why we decided to investigate the influence of GNRA-motive alterations in apical loop of IV domain of Coxsackie virus B3 IRES on expression levels alone without secondary structure disruption.

Using NCBI database we analyzed the sequences of different wild-type IRESes of Coxsackie viruses. We found that, there are only three types of natural GNRA-motives in this position: GCGA, GTAA, and GTGA[3]. We chose the IRES of Coxsackie virus B3, containing GCGA-motif (CVB3-GCGA) and made two mutant-IRESes: CVB3-GTAA and CVB3-GTGA. Then, to ensure that secondary structure stayed same, we visualized the predicted structure of three IRESes, using the Vienna RNA Websuite[2].We obtained three constructions of circular RNA expressing firefly luciferase. After *in vitro* transcription, cyclization and products purification we performed a transfection of HEK-293 cells using Lipofectamine 2000 with subsequent luciferase activity measurement.

Comparing to normal expression of firefly luciferase by wild-type CVB3-GCGA, CVB3-GTAA showed ∼50% reduction, while CVB3-GTGA ∼75% reduction. This difference may be explained by the existence of complex interactions of different domains, caused by a number of compensatory mutations in other parts of wild-type IRESes, containing different GRNA-motives. Hence, the alteration of only one part of these mechanism lead to a significant loss of activity. Nevertheless, further investigation is required for deep understanding of these mechanisms.

References

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